Vol. 110. Part 1. Pp. 1–20. 22 figs. June 3rd, 1958.

COMMONWEALTH INST. OF ENTOMOLOGY LIBRARY
- 5 JUN 1958
Eu. 3HH

# THE TRANSACTIONS OF THE ROYAL ENTOMOLOGICAL SOCIETY OF LONDON

*World List* abbreviation: *Trans. R. ent. Soc. Lond.*

## CONTENTS

PAGE

SINGH, T. Ovulation and corpus luteum formation in *Locusta migratoria migratorioides* Reiche and Fairmaire and *Schistocerca gregaria* (Forskål) . 1–20. 22 text-figs.

LONDON:
PUBLISHED BY THE SOCIETY AND
SOLD AT ITS ROOMS, 41, QUEEN'S GATE, S.W.7.

*Price 7s. 6d.*

# THE ROYAL ENTOMOLOGICAL SOCIETY OF LONDON

**Founded 1833. Incorporated by Royal Charter 1885.**

**Patron:**
HER MAJESTY THE QUEEN.

## OFFICERS AND COUNCIL FOR THE SESSION, 1958–1959

PROF. O. W. RICHARDS, *President.*
MR. E. B. BRITTON, MR. W. V. HARRIS, DR. H. E. HINTON } *Vice-Presidents.*
DR. N. E. HICKIN, *Treasurer.*
MR. PAUL FREEMAN, *Secretary.*
MR. W. H. POTTS, *Editor.*

*Other Members of Council.*

DR. C. G. BUTLER.
DR. L. G. HIGGINS.
DR. B. M. HOBBY.
MR. T. G. HOWARTH.
DR. J. S. KENNEDY.
DR. A. M. MASSEE, O.B.E.
MR. E. O. PEARSON.
MR. H. L. G. STROYAN.
DR. N. WALOFF.
DR. C. B. WILLIAMS, F.R.S

MISS E. EVANS, *Registrar.*

*Finance and House Committee.*
MR. H. L. G. STROYAN (Chairman).

DR. J. R. BUSVINE.
DR. C. G. BUTLER.
DR. R. M. GREENSLADE.
DR. A. G. HAMILTON.
MR. A. T. THOMPSON.

*Publication and Library Committee.*
DR. B. M. HOBBY (Chairman).

MR. R. F. BRETHERTON, C.B.
DR. W. J. HALL, C.M.G., M.C.
DR. H. E. HINTON.
MR. T. G. HOWARTH.
MR. H. OLDROYD.

*Committee for the Protection of British Insects.*
MR. H. M. EDELSTEN, O.B.E. (Chairman).

MR. R. B. BENSON.
MR. R. F. BRETHERTON, C.B.
DR. B. M. HOBBY (*and representing the Society for British Entomology*).
CAPT. R. A. JACKSON, C.B.E., R.N.
LIEUT.-COL. W. B. L. MANLEY.
DR. A. M. MASSEE, O.B.E.
DR. N. W. MOORE.
MR. A. ROEBUCK, O.B.E.
MR. N. D. RILEY, C.B.E. (Secretary).

The Executive Officers of the Society are *ex-officio* members of all Committees.

## DELEGATES OF THE SOCIETY TO:

1. **British National Committee for Biology** (Royal Society).
   *Mr. N. D. Riley, C.B.E., appointed* 1955.
   *Dr. H. E. Hinton, appointed* 1958.
2. **Local Committee of Management of Wicken Fen.**
   *Mr. H. M. Edelsten, O.B.E.* (nominated by Committee for the Protection of British Insects).
3. **National Trust for Places of Historic Interest or Natural Beauty.**
   *Mr. N. D. Riley, C.B.E., appointed* 1955.
4. **New Forest Association.**
   *Mr. C. W. Mackworth-Praed, appointed* 1948.
5. **New Forest Joint Committee.**
   *Lt.-Col. F. C. Fraser, appointed* 1949.
6. **Field Studies Council.**
   *Dr. W. H. Thorpe, F.R.S., appointed* 1944.
7. **Yorkshire Naturalists' Trust, Ltd.**
   *Dr. W. D. Hincks, appointed* 1946.
8. **The Biological Council.**
   *Dr. N. E. Hickin, appointed* 1952.
9. **The Parliamentary and Scientific Committee.**
   *Dr. N. E. Hickin, appointed* 1953.
10. **British Co-ordinating Committee for Nature Conservation.**
    *Mr. E. B. Britton, appointed* 1954.

# OVULATION AND CORPUS LUTEUM FORMATION IN *LOCUSTA MIGRATORIA MIGRATORIOIDES* REICHE AND FAIRMAIRE AND *SCHISTOCERCA GREGARIA* (FORSKÅL)

By T. Singh

Manuscript received 8th July, 1957

(Read 6th November, 1957)

With 22 Text-figures

Contents


| | | PAGE |
|---|---|---|
| I. | Introduction | 1 |
| II. | Technique | 2 |
| III. | *Locusta migratoria migratorioides* R. and F. | |
| | (1) Description of adult ovary | 3 |
| | (2) Oviposition | 3 |
| | (3) Anatomy of the corpus luteum and its pigmentation | 6 |
| | (4) Histology of corpus luteum | 11 |
| | (*a*) Histology of first corpus luteum | 11 |
| | (*b*) Degeneration of follicle cells | 11 |
| | (*c*) Histology of a second corpus luteum | 14 |
| | (*d*) Histology of the third and successive corpora lutea | 14 |
| | (5) Degenerate ovarioles and the formation of resorption bodies | 18 |
| IV. | *Schistocera gregaria* (Forsk.) | 18 |
| V. | Summary | 19 |
| VI. | Acknowledgments | 20 |
| VII. | References | 20 |


## I. Introduction

Dissection of mature female insects frequently shows the presence at the base of one or more ovarioles of structures which are sometimes of a yellow-orange colour and are known as the "corpora lutea". These have been reported in several orders of insects but the literature on their structure is very limited, though many papers on the reproductive organs contain incidental information. Among these may be mentioned the following :—

Ahrens (1935) observed in *Termes redemanni* a colourless corpus luteum formed successively by the first, second and often third egg follicles and some light yellow pigmented bodies in the distal cells of the peduncle. Wigglesworth (1936) observed after discharge of an egg in *Rhodnius prolixus*, a corpus luteum the cells of which undergo necrosis and absorption and by the time the next oocyte is ripe the preceding follicle disappears. Vel'tischev (1941) recorded

corpora lutea in *Locusta migratoria* and believed them to indicate that ovulation had occurred. Roonwal (1947) noted the occurrence of a mass of orange-red pigment lying in the small basal follicle or peduncle in *Schistocerca gregaria*. Palm (1948) studied *Bombus* and *Psithyrus* and noted in the former that the corpus luteum is formed by the nurse-follicle epithelium, which absorbs the necrotic egg-follicle epithelium. In pathological cases when a large oocyte degenerates and is resorbed a similar process occurs in the egg-follicle epithelium. No pigment was observed in the corpus luteum and he points out that the name is misleading. De Lerma (1951) observed a yellow corpus luteum in *Locusta danica* (= *L. migratoria*), formed by the degeneration of the follicular mass, which is ultimately resorbed. Bonhag and Wick (1953) observed an unpigmented corpus luteum in *Oncopeltus fasciatus* after the discharge of the egg. Later the contents of the corpus luteum are discharged and the old follicle shrinks to an inconspicuous structure.

Apart from the detailed cytological work of Ahrens, however, the authors cited above have not provided a very full account of the processes involved in corpus luteum formation. It is also not clear whether pigment is always deposited or whether, when it is present, it always develops in the same place. It is uncertain from the literature to what extent the presence of corpora lutea is proof of ovulation having taken place and whether it is possible to ascertain, from the presence of multiple corpora lutea, how many eggs have been discharged from the ovarioles. For these reasons, and to extend as far as possible our knowledge of the formation of corpora lutea, it was decided to investigate the subject in detail.

In this paper it is proposed to deal with *Locusta* in detail and with *Schistocerca* briefly. Further papers in preparation will deal with *Dysdercus fasciatus* Sign. and *Ephestia kühniella* Zell.

## II. Technique

*Locusta migratoria*.—Last-instar nymphs were obtained from the Imperial College Field station, Silwood Park, Berks., and reared in cylindrical celluloid breeding cages kept in a constant temperature room (28–32° C. and 65–75 per cent. R.H.). Newly-emerged adults were separated into pairs, a male and female of the same age being kept in each cage. A correct record of the age of the female was kept.

*Schistocerca gregaria*.—These were reared in the same way as *Locusta*, but at 36° C. and 55 per cent. R.H.

### *Fixation and Microscopical Technique*

Female locusts were narcotised in ether and dissected under Bouin's fluid. They were dissected when two, four and six days old and thereafter were dissected at daily intervals in order to study the progressive development of the ovarioles. Immediately after dissection the ovarioles were transferred to fresh Bouin's fluid. Five to 6 $\mu$ sections were cut and stained with Heidenhain's iron haematoxylin or Ehrlich's haematoxylin and counter-stained with eosin in 90 per cent. alcohol. Mallory's Triple Stain (Pantin, 1948) was also used. Each of these stains had its own advantages, the last being valuable for differentiating the cellular structures from the *tunica propria*.

## III. *Locusta migratoria migratorioides* R. & F.

### (1) *Description of Adult Ovary*

The ovaries, which are enclosed in a tough fenestrated cellular membrane, are whitish translucent bodies in an immature adult up to the sixth day, but on the eighth day the basal egg of each ovariole becomes yellow, due to yolk-deposition, and imparts a yellow colour to the ovaries. In mature females the fenestrated membrane becomes weaker and can be easily broken. Each ovary, which is composed of 85–105 panoistic ovarioles, progressively increases in length and measures, on the average, 18 mm. at full development on the ninth day after emergence of the adult. Each ovariole increases in length as it grows from 3 mm. to 8·5 mm. and is disposed obliquely to the lateral oviduct. It consists of an elongated tube distinguishable into three regions : (*a*) *Terminal filament*, a slender, thread-like region that forms the anterior part of the ovariole; (*b*) *germarium*, a thicker short region below the terminal filament where the egg rudiments are not differentiated ; and (*c*) *vitellarium*, a region beyond the germarium having a chain of developing oocytes. In the vitellarium the number of visible egg rudiments first increases progressively from 12 to 22, but at the approach of maturity it becomes constant until oviposition starts. The oldest egg lies at the base of the ovariole and is recorded as egg 1. The terminal filaments of the ovarioles unite to form a median ligament. The two ligaments are closely adherent, uniting the two ovaries. Each ovariole is united to a muscular tube, the lateral oviduct, by a short pedicel which grows from 0·1 to 0·2 mm. between the second and ninth days after emergence. The lateral oviduct of each side leads into a short common oviduct. Anteriorly each lateral oviduct ends in a tubular accessory gland, simple at first but forming a convoluted glandular structure by the ninth day. By following the rate of development of egg 1 and egg 2 it can be shown that the rate of development of egg 1 is much accelerated from the seventh day onwards, while that of egg 2 also increases, though not so much as egg 1, at the approach of ovulation. Egg 2 remains colourless until egg 1 is discharged.

### (2) *Oviposition*

A tube of sand was provided in each cage for 25 separate pairs of males and females of known ages after the females had attained the age of six days. The sand-tubes were examined every morning and evening for newly-laid pods and the previous day's sand was replaced by fresh moist sand. The maturation time of 25 females is given in Table I, from which it is evident that the normal

TABLE I

| Days after emergence | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of females laying first pod | 1 | 4 | 4 | 3 | 3 | 3 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |

maturation time for laying a pod is between 9 and 16 days. Those laying a pod after 16 days were regarded as abnormal.

A dozen cages were again set up, each with a male and female of known ages, and females were dissected within 24 hours after the first pod had been laid. In several cases the dissection was made immediately after oviposition

was completed. The pod was taken from the sand and the eggs counted. Furthermore, the ovarioles of the particular insect which had laid a pod were also counted. It was observed that in some cases the total number of ovarioles did not correspond to the number of eggs in a pod but the number of eggs corresponded exactly with the number of ovarioles having either a colourless or pigmented corpus luteum, hereinafter designated as "ovulated". The other ovarioles were of two types—some were colourless and much smaller in size with a much smaller basal egg and an orange-red structure resembling a corpus luteum (fig. 1); the remainder had a basal egg slightly shorter than the "ovulated" ones with yellow yolk and a crescentic zone of pigment at the base in the region of the plug (fig. 2). Both these types were classified as "unovulated". Details of the count are given in Table II; attention is specially drawn to specimens C, D, F and I, in which no ovarioles fell into the "unovulated" category and the number of eggs was identical with that of ovarioles.

TABLE II.—*Relation of number of eggs in a pod to the number of ovulated ovarioles*

| Insects | Days after emergence first pod laid | No. of eggs in first pod | Ovarioles | | |
|---|---|---|---|---|---|
| | | | "Ovulated" | "Unovulated" | Total |
| A | 10 | 100 | 100 | 3 | 103 |
| B | 10 | 100 | 100 | 5 | 105 |
| C | 10 | 96 | 96 | 0 | 96 |
| D | 10 | 99 | 99 | 0 | 99 |
| E | 9 | 100 | 100 | 5 | 105 |
| F | 11 | 94 | 94 | 0 | 94 |
| G | 9 | 101 | 101 | 2 | 103 |
| H | 10 | 99 | 99 | 5 | 104 |
| I | 10 | 100 | 100 | 0 | 100 |
| J | 10 | 96 | 96 | 4 | 100 |
| K | 9 | 97 | 97 | 2 | 99 |
| L | 10 | 101 | 101 | 3 | 104 |

It can therefore be concluded that at each act of oviposition only one egg is normally ovulated by each ovariole, but that often a few ovarioles do not produce an egg.

In ten insects successive pods were collected and a count of the number of eggs in each pod was made. The interval between the laying of successive pods was also recorded. The results are tabulated in Table III, where the upper figures indicate the age of the female (in days from emergence) when the pod was laid and the lower figures (in brackets) the numbers of eggs in the pod. The insects were dissected before the end of their reproductive life and the number of ovarioles found is also given in Table III.

From Table III it is clear that the interval between successive ovipositions varies from two to six days, with an average of about 3·9 days. Also, the number of eggs in successive pods is not constant nor does it decline continuously throughout life; later pods sometimes have more eggs than preceding ones.

A further understanding of the variability in egg counts was obtained by examining the various categories of ovarioles present on dissection of the females

TABLE III.—*Age of adult females when successive pods are laid and numbers of eggs per pod*

| Insects | 1st pod | 2nd pod | 3rd pod | 4th pod | 5th pod | 6th pod | 7th pod | Total number of ovarioles |
|---|---|---|---|---|---|---|---|---|
| A | 11 | 17 | 21 | 26 | 30 | 33 | . | 90 |
| | (66) | (83) | (83) | (82) | (83) | (85) | | |
| B | 11 | 15 | 18 | 23 | 26 | . | . | 78 |
| | (56) | (57) | (57) | (57) | (52) | | | |
| C | 13 | 16 | 19 | 23 | 29 | 32 | 35 | 112 |
| | (83) | (71) | (87) | (89) | (88) | (82) | (82) | |
| D | 14 | 19 | 23 | 27 | . | . | . | 94 |
| | (89) | (86) | (89) | (89) | | | | |
| E | 15 | 19 | 22 | . | . | . | . | 97 |
| | (85) | (85) | (84) | | | | | |
| F | 14 | 19 | 24 | 29 | 35 | . | . | 85 |
| | (59) | (40) | (39) | (42) | (49) | | | |
| G | 14 | 16 | 19 | . | . | . | . | 88 |
| | (89) | (82) | (82) | | | | | |
| H | 11 | 13 | 16 | 18 | 21 | . | . | 112 |
| | (94) | (99) | (96) | (99) | (96) | | | |
| I | 13 | 16 | 19 | . | . | . | . | 100 |
| | (96) | (96) | (96) | | | | | |
| J | 11 | 13 | 16 | 20 | 24 | 29 | 34 | 100 |
| | (94) | (96) | (96) | (93) | (96) | (96) | (93) | |

whose history is given in Table III. The results are given in Table IV, where the figures for " normal ovarioles " are averages for 20 ovarioles. The others are averages based on the number of ovarioles in the appropriate category, defined as follows:

*Ovulated*

(i) *Normal:* ovarioles undergoing normal development always have a white corpus luteum at the base after ovulation (fig. 3).

(ii) *Subnormal:* ovarioles which were slightly shorter in length than the normal and in which the length of the basal egg was also slightly less and an orange-yellow pigment was either uniformly distributed over the whole corpus luteum (fig. 4) or formed a crescentic ring of pigment at the junction of the basal egg and the corpus luteum (fig. 5). In some subnormal ovarioles the corpus luteum was seen to be highly pigmented and contracted and the long basal egg possessed a yellowish-orange pigment easily confused with that of a normal yolky egg.

*Unovulated*

(i) *Partially degenerate:* ovarioles whose length was much shorter than the normal or subnormal ovarioles. The visible egg rudiments were much fewer and the size of the basal egg was also much reduced with a rounded (fig. 1) or crescentic yellowish-orange pigmented body (fig. 2) in the region of the plug. In some cases two shades of pigments were noticed, orange-yellow and deep orange, appearing as if the degeneration had occurred in two successive stages. The lower faint orange-yellow stage was delimited from the upper deep orange region by a constriction (fig. 6).

(ii) *Completely degenerate :* ovarioles which were totally devoid of egg rudiments though a small pigmented mass was present at the base of the region of the plug (fig. 7).

TABLE IV.—*Categories of ovarioles after successive pods*

| | | Ovulated | | | | | | | | Unovulated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Normal | | | | Subnormal | | | | Partially degenerate | | | | Completely degenerate | |
| Insects | Total no. of ovarioles | No. present | Length of ovarioles (mm.) | No. of visible rudiments | Length of basal egg (mm.) | No. present | Length of ovarioles (mm.) | No. of visible rudiments | Length of basal egg (mm.) | No. present | Length of ovarioles (mm.) | No. of visible rudiments | Length of basal egg (mm.) | No. present | Leng of ovar iole (mm |
| A . | 90 | 80 | 7·5 | 21 | 2·5 | 5 | 7·18 | 21 | 2·25 | 1 | 5·0 | 15 | 1·25 | 4 | 3·( |
| B . | 78 | 50 | 7·6 | 21 | 2·6 | 2 | 7·4 | 20 | 2·4 | 11 | 5·2 | 14 | 1·0 | 15 | 2·5 |
| C . | 112 | 79 | 6·6 | 19 | 1·9 | 3 | 6·2 | 19 | 1·7 | 8 | 4·0 | 12 | 0·9 | 22 | 2·( |
| D . | 94 | 87 | 7·9 | 21 | 3·0 | 2 | 7·5 | 21 | 2·5 | 2 | 5·2 | 15 | 1·0 | 3 | 2·3 |
| E . | 97 | 83 | 7·6 | 20 | 2·5 | 1 | 7·2 | 20 | 2·3 | 5 | 5·0 | 13 | 1·0 | 8 | 2·( |
| F . | 85 | 46 | 7·7 | 21 | 2·5 | 3 | 7·5 | 21 | 2·4 | 15 | 5·1 | 15 | 1·2 | 21 | 2·2 |
| G . | 88 | 80 | 7·6 | 21 | 2·4 | 2 | 7·5 | 21 | 2·1 | 3 | 5·2 | 16 | 1·1 | 3 | 3·( |
| H . | 112 | 94 | 6·8 | 20 | 2·6 | 2 | 6·3 | 20 | 2·3 | 8 | 4·5 | 14 | 0·9 | 8 | 2·5 |
| I . | 100 | 95 | 7·5 | 21 | 3·0 | 1 | 7·1 | 21 | 2·5 | 1 | 5·6 | 16 | 1·0 | 3 | 2·3 |
| J . | 100 | 90 | 7·3 | 19 | 2·9 | 3 | 7·1 | 19 | 2·4 | 2 | 5·0 | 14 | 1·1 | 5 | 2·2 |
| Average . | 95·6 | 78·4 | 7·31 | 20·4 | 2·59 | 2·4 | 7·09 | 20·3 | 2·28 | 5·6 | 4·98 | 14·4 | 1·04 | 9·2 | 2·4 |

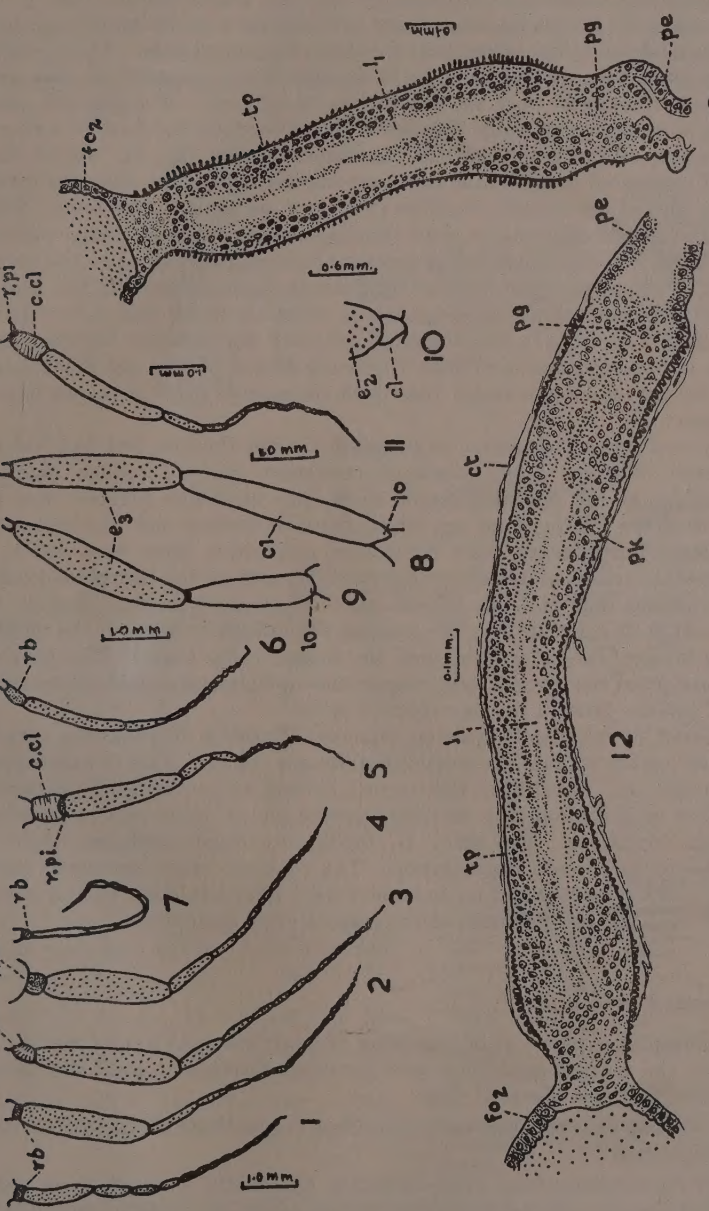
It is clear that the lower number sometimes found in some females is not due to failure of an otherwise normal ovariole to mature an egg, since counts show that the number of eggs laid in a pod always corresponds exactly with the number of ovarioles which have ovulated and either possess colourless (normal) or pigmented (subnormal) corpora lutea. Variation in the number of eggs in successive pods is probably due to the partially degenerate ovarioles attaining normal activity in a succeeding ovulation, while, conversely, some normal ovarioles probably undergo partial or complete degeneration after ovulating. It may reasonably be concluded that the degeneration is not an irreversible feature in the reproductive cycle.

### (3) *Anatomy of the Corpus Luteum and its Pigmentation*

Immediately after ovulation, when the egg has just left its follicle, the latter consists of an empty colourless tube averaging about 3·2 mm. long (fig. 8),

---

FIGS. 1–13.—(1) Partially degenerate ovariole showing pigmented resorption body and immature basal oocyte. (2) Partially degenerate ovariole showing mature basal oocyte and pigmented remains of resorption body at the base. (3) Normal ovariole within 24 hours of ovulation, showing colourless corpus luteum at the base. (4) Ovariole within 24 hours of first oviposition, showing pigmented corpus luteum and shorter basal oocyte. (5) Ovarioles within 24 hours of first oviposition showing colourless corpus luteum, a ring of pigment at the top and slightly shorter basal oocyte. (6) Degenerate ovariole, showing resorption of two oocytes forming a pigmented resorption body. (7) Completely degenerate ovariole, showing pigmented resorption body at the base. (8) Basal part of ovarioles, immediately after second ovulation, showing contraction of corpus luteum. (9) Basal part of ovarioles, immediately after second ovulation, showing further contraction of corpus luteum. (10) Basal part of ovariole one day after first pod, showing contraction of corpus luteum. (11) Ovariole showing colourless corpus luteum and a pigmented ring at the base, after second ovulation. (12) L.S. through follicle immediately after ovulation, showing slight contraction of follicle to form tubular corpus luteum. (13) L.S. through tubular corpus luteum in second stage of contraction.




For explanation see opposite page.
Explanation of lettering on page 20.

which gradually contracts to 0·4 mm. by 24 hours after ovulation (figs. 9, 10), thus forming the corpus luteum. Some ovarioles have colourless corpora lutea and others (the so-called subnormal type) have pigmented ones. The colourless corpora lutea were at first thought to develop orange pigment as they grew older, but subsequent work showed this to be an error. The pigment, when present, was either uniformly distributed (fig. 4) or took the form of a ring at the junction of the basal egg and the corpus luteum (fig. 5). In all these cases of pigmented ovarioles the basal egg was slightly shorter than the corresponding one in the normal ovarioles (2·28 mm. as against 2·59 mm.; Table IV). After several ovipositions, some ovarioles possess yellowish-orange pigment in the fairly well developed but degenerate basal egg as well as in the corpus luteum. Earlier, the same type of pigment was also observed at the base of the partially and completely degenerated ovarioles which had not ovulated at all (figs. 1, 6, 7). It was therefore felt that pigmentation of the corpus luteum is perhaps associated with the degeneration of the egg immediately above the corpus luteum rather than with the normal cycle of corpus luteum development.

As described in the section on oviposition, after the first pod was laid the degenerate ovarioles each possess a crescentic zone of orange pigment. Presumably, as the recorded counts show, this crescentic pigment was the remnant of the follicle of an egg which failed to mature and had undergone resorption. Furthermore, after the second oviposition there were insects in which some ovarioles possessed a crescent of pigment at the base of a colourless corpus luteum (fig. 11). In several cases where analysis was possible the number of such crescents was the same as the difference between the number of eggs in the first and second pod, the former being lower. This provides additional proof that the partially degenerate ovarioles are capable of resuming normal activity later in the reproductive cycle.

In order to investigate whether pigment deposition in colourless corpora lutea ever occurs without degeneration of the egg above it, a set of experiments were carried out. The object was to run a control set providing all the normal conditions of food, humidity and temperature and in other sets to introduce abnormal conditions. The latter, by tending to inhibit ovulation might be expected to accentuate degeneration. The ovarioles were examined within 24 hours of oviposition and on successive days after ovulation until a second pod was laid and the condition of the corpora lutea examined.

*Experiment* 1

A controlled experiment of four pairs of newly emerged locusts was set up in which the normal conditions were provided throughout until the second oviposition. It was observed that:

(1) Normal ovarioles possess a colourless corpus luteum up to four days after oviposition.

(2) Of the ovarioles from which ovulation apparently occurred, those which developed pigment were few in number and their basal eggs ($egg_2$) were markedly smaller than the corresponding egg of the ovarioles with colourless corpora lutea (Table V).

(3) In ovarioles where $egg_2$ was degenerating the crescent-shaped ring of pigment (insect B) was deposited at the upper end of the colourless corpus luteum (fig. 5), or in some cases (insect A) throughout the corpus luteum (fig. 4). It is not clear whether one of these forms is a stage in the development of the other.

TABLE V

| Insects | Days after first oviposition | No. of eggs in the pod | No. of ovarioles | No. of corpora lutea | No. of pigmented corpora lutea | No. of degenerate ovarioles with pigmented base | Average length of basal egg in ovarioles having colourless corpora lutea (mm.) | Average length of basal egg in ovarioles having pigmented corpora lutea (mm.) |
|---|---|---|---|---|---|---|---|---|
| A | 1 | 90 | 99 | 90 | 10 | 9 | 2·25 | 1·9 |
| B | 1–2 | 87 | 101 | 87 | 3 | 14 | 2·65 | 2·5 |
| C | 2–3 | I pod 82<br>II pod 87 | 89 | 82+5 | 0 | 2 | 3·6 | . |
| D | 3–4 | 76 | 92 | 76 | 10 | 16 | 3·9 | 3·65 |

TABLE VI

| Insects | Days after first oviposition | No. of eggs laid | No. of ovarioles | No. of corpora lutea | No. of pigmented corpora lutea | No. of degenerate ovarioles: With pigmented base | No. of degenerate ovarioles: With two shades of pigmented base | No. of degenerate ovarioles: With pigmented base and pigmented basal egg | Length of basal egg in ovarioles having colourless corpus luteum (mm.) | Length of basal egg in ovarioles having pigmented corpus luteum (mm.) |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 1 | 92 | 96 | 92 | 13 | 4 | 0 | 0 | 3·5 | 3·25 |
| B | 2 | 88 | 108 | 88 | 0 | 14 | 4 | 2 | 3·0 | . |
| C | 3 | I pod 79<br>II pod 84 | 113 | 79+5 | 0 | 16 | 4 | 9 | 2·75 | . |
| D | 4 | 90 | 107 | 90 | 9 | 2 | 3 | 12 | 2·5 | 2·2 |

TABLE VII

| Insects | Day after first oviposition | No. of eggs laid | No. of ovarioles | No. of corpora lutea | No. of pigmented corpora lutea | No. of degenerate ovarioles with pigmented base | Length of basal egg in ovarioles having colourless corpus luteum | Length of basal egg in ovarioles having pigmented corpus luteum (mm.) |
|---|---|---|---|---|---|---|---|---|
| A | 1 | 61 | 80 | 61 | 52 (*a*)<br>9 (*b*) | 10 | . | 3·2 |
| B | 2 | 74 | 103 | 74 | 70 (*a*)<br>4 (*b*) | 29 | . | 3·0 |
| C | 3 | 73 | 95 | 73 | 67 (*a*)<br>6 (*b*) | 22 | . | 3·2 |
| D | 4 | I pod 97<br>II pod 45 | 109 | 45 | 12 (*c*) | | | |

All ovarioles have pigment at the base. It is difficult to divide them into categories.

(*a*) Corpora lutea having a ring of pigment at their distal ends.
(*b*) Fully pigmented corpora lutea.
(*c*) Completely degenerate ovarioles having no egg rudiments but pigmented base present.

(4) In insect C crescent-shaped pigment was seen at the base of five colourless corpora lutea in five ovarioles after the second ovulation. Egg counts also show that this number is the difference between the number of eggs in the first and second pods, the former being lower. Therefore it could be concluded that five ovarioles which failed to mature an egg in the first ovulation, *i.e.*, whose first egg was resorbed, became normal in the second ovulation and the crescentic zone of pigment is the result of resorption of the preceding egg. The size of the basal egg ($egg_3$) in those five ovarioles being identical to the corresponding size of the basal egg ($egg_3$) of the ovarioles with colourless corpora lutea provides additional proof that partially degenerate ovarioles are capable of resuming normal activity later in the reproductive life.

(5) Partially or completely degenerate ovarioles which, to judge from pod counts, did not ovulate always have a deeply pigmented corpus luteum at the base.

*Experiment* 2

In four pairs of newly emerged locusts normal conditions were provided until first oviposition ; later they were kept at a lower temperature (28–29° C.). The other factors remained the same as in the first experiment. It was observed that :

(1) The corpus luteum of a normal ovariole remains colourless as in Experiment 1, although compared with that experiment there is a slight increase in the number of degenerate ovarioles (Table VI).

(2) If the normal temperature (32–35° C.) is not provided, $egg_2$ undergoes degeneration. The corpus luteum develops deep orange pigment and $egg_2$ acquires a yellowish-orange colour.

(3) In degenerate ovarioles succeeding eggs undergo further resorption and impart different shades of colour to the respective corpora lutea (fig. 6).

(4) Again in this set (Table VI, insect C) five degenerate ovarioles resumed normal activity in the second ovulation, as observed in Experiment 1.

*Experiment* 3

In this experiment recently emerged locusts were kept under normal conditions until the first pod was laid ; later no food was given but the other factors were kept as in the control experiment. No colourless corpora lutea were noted in this series (Table VII). Proof that the origin of the pigment is associated with the degeneration of the egg, which has now assumed the basal position ($egg_2$), is difficult to obtain in this series since they are not noticeably reduced in size and there are no unpigmented ovarioles to make possible a comparison. The fact that pigmentation appears in the egg concerned, however, together with the results of the earlier experiments, make the hypothesis not unreasonable.

Summarising the more important results of the experimental work of this section, one may say that the appearance of pigment at the base of the ovarioles is not a normal feature of corpus luteum formation in *Locusta*. Under optimal environmental conditions the normal ovariole forms a colourless corpus luteum after ovulation, but a small proportion of the ovarioles from which ovulation has occurred develop various degrees of pigmentation and this is correlated

with a reduction in the length of the egg due next to be ovulated. The pigment may therefore be supposed to arise in connection with a resorptive process in this egg, and the withdrawal of food after oviposition, which it is plausible to suppose affects the maturation of the succeeding eggs, also invariably causes pigmentation of the corpora lutea formed after that oviposition.

### (4) *Histology of Corpus Luteum*

#### (a) *Histology of First Corpus Luteum*

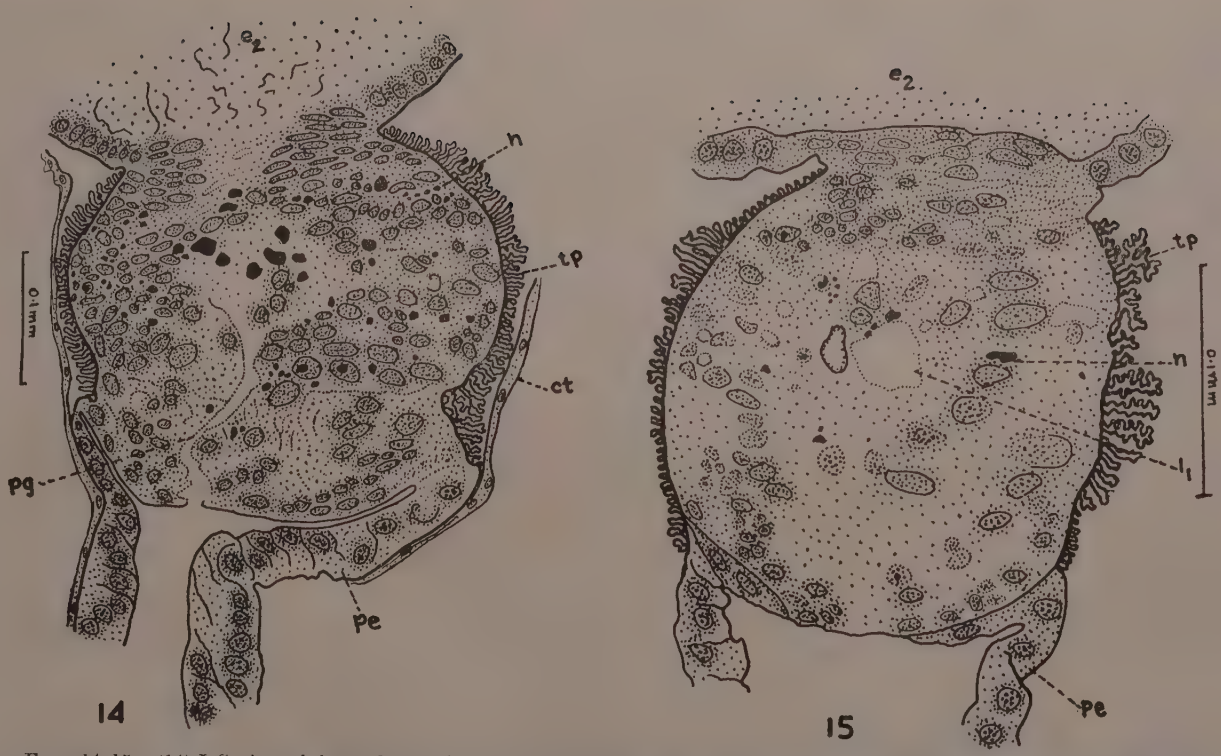
In a fully mature follicle the nuclear material is broken up into a large number of deeply basophilic granules and the nuclear membrane is quite prominent. By this time the chorion is laid down all over the egg. At the time of ovulation the epithelial plug breaks down and the egg in the basal follicle enters the lateral oviduct. The follicle anterior to the discharged follicle then becomes the basal follicle and the basal inter-follicular plug assumes the function and position of the epithelial plug.

After the discharge of the first egg from the follicle, the wall of the follicle gradually contracts, producing a simple tubular first corpus luteum the cells of which are seen in a rapid state of degeneration (fig. 12). The folding of the *tunica propria* is an indication of the gradual contraction of the follicle. More folds are seen at the proximal and distal ends of the empty follicle. These folds gradually increase along the whole length following further contraction of the follicle (fig. 13), and the tubular corpus luteum consequently shortens (fig. 14). At the end of the contraction the folds of the *tunica propria* become more conspicuous because of secondary foldings. In spite of the considerable contraction of the egg space no crumpling of the follicle epithelium takes place. The epithelium is not separated from the *tunica propria* and there is no folding of the wall.

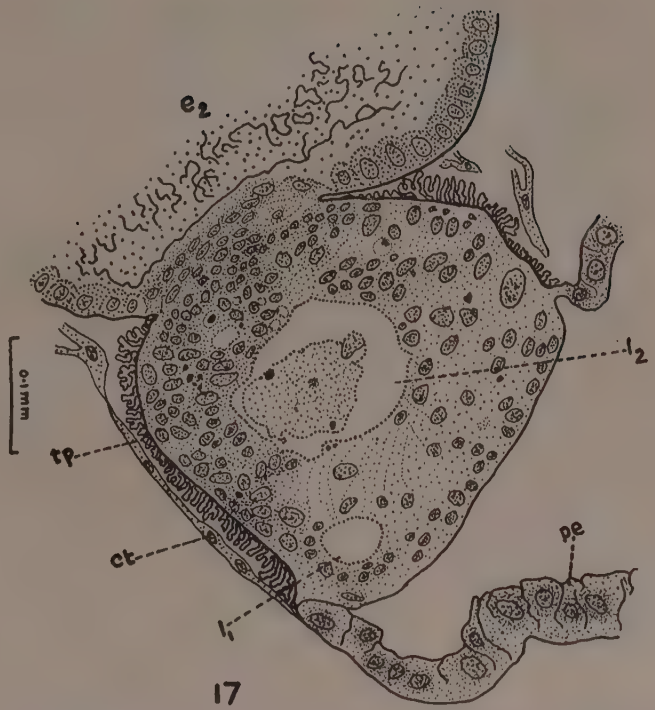
Within 24 hours the cells of the corpus luteum undergo pyknotic degeneration. The structure becomes quite compact and a proximal end, showing the site of passage of the egg, may be distinguished from a distal end with large, pyknotic, autolysing nuclei (fig. 14). At this time the cavity of the corpus luteum becomes reduced, while the pyknotic nuclei become few in number and less basophilic. The egg passage is then no longer visible and finally the folded *tunica propria* become more complicated because of secondary foldings (fig. 15).

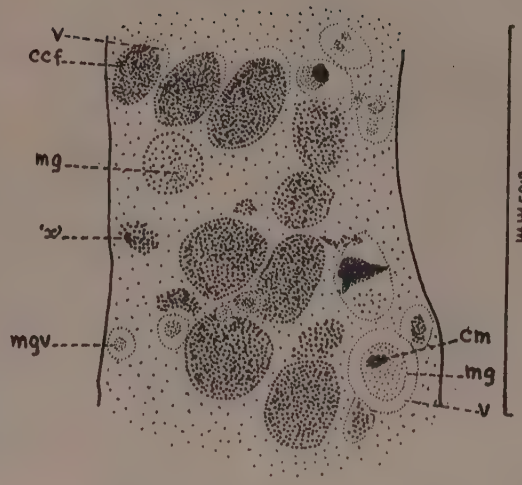
#### (b) *Degeneration of Follicle Cells*

The earlier signs of degeneration are clearly seen in the cytoplasm, where the cellular nature of the follicle is completely disturbed and conspicuous changes occur in the nucleus. The basophilic chromatin granules swell and each nucleus enlarges. In some cases the nuclear membrane bursts, liberating the contained chromatin granules into the surrounding cytoplasm (fig. 16). These free granules may appear reduced in number and stain less heavily in haematoxylin. Alternatively, the nuclear membrane does not disrupt, but remains to enclose a vacuole after the disappearance of the chromatin granules. This disappearance of the granules can be traced through successive stages in which their number diminishes and they become less basophilic. In the




FIGS. 14–15.—(14) L.S. through base of ovariole within 24 hours after laying of first pod, showing contraction of corpus luteum, a passage of the egg, follicle cells undergoing pyknosis and folding of the tunica propria. (15) L.S. through base of ovariole one day after laying of first pod, showing few pyknotic nuclei and conspicuous lumen of first follicle.




FIGS. 16–17.—(16) L.S. of portion of follicular wall from fourth stage of contraction of corpus luteum, showing stages of pyknosis and karyolysis. (17) L.S. through base of ovariole within 24 hours after laying second pod, showing degenerating lumen of first follicle and well developed lumen of second follicle forming second corpus luteum.

second case the contents of the large follicle cell nuclei break up into conspicuous basophilic granules which sometimes aggregate into irregular rods. After the absorption of some of this heavily staining chromatin material, the remainder forms a compact homogeneous dark mass in a vacuole. Eventually the strong basophilic reaction of this body is lost, when it appears as a mauve or pinkish globule within the surrounding vacuole. The progressive stages of this transformation can be observed in different cells in which variable portions of the globule have lightened to mauve, leaving the remainder dark. Finally this mauve globule disappears, sometimes leaving an empty vacuole or no trace.

The absorption of the fluid contents of the nucleus can probably occur in two ways—(1) sudden disintegration, known as " necrosis ", when the process occurs abruptly and the nuclear solids are compacted into a single mass, and (2) gradual disintegration known as " necrobiosis ", where gradual absorption results in the formation of discrete chromatic granules. In the necrotic type of nuclear disintegration, the condensation of chromatin material to form a dark compact mass is termed " pyknosis ". The following events, resulting in the steady disappearance of the dark staining chromatin material through a pale globule stage, as described above, to its final disappearance are termed " karyolysis " (Wright, 1954).

(c) *Histology of a Second Corpus Luteum*

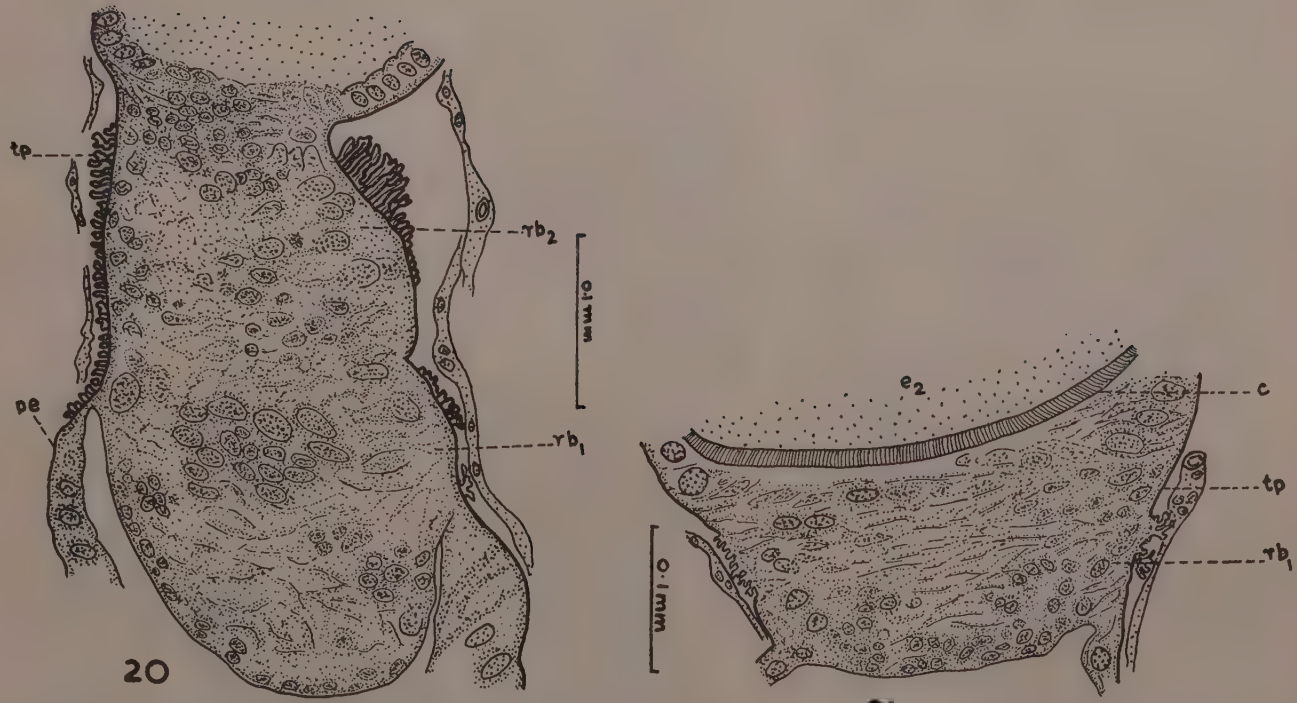
When the second egg is fully mature the interfollicular wall bulges into the lumen of the first corpus luteum and subsequently bursts, allowing the second egg to be pushed down through the remains of the preceding corpus luteum, which by this time is much reduced (fig. 17). The second corpus luteum is then formed in a manner similar to the first, so that two corpora lutea are visible in the compound structure lying at the base of the ovariole. The proximal part of this structure, with weakly basophilic pyknotic nuclei, represents the remnant of the first corpus luteum enclosing the small cavity (fig. 17) of the first follicle, while the distal part, with deeply basophilic pyknotic nuclei, encloses the large cavity of the second follicle. During this contraction the *tunica propria* folds still more and forms a thick convoluted mass externally.

(d) *Histology of the Third and Successive Corpora Lutea*

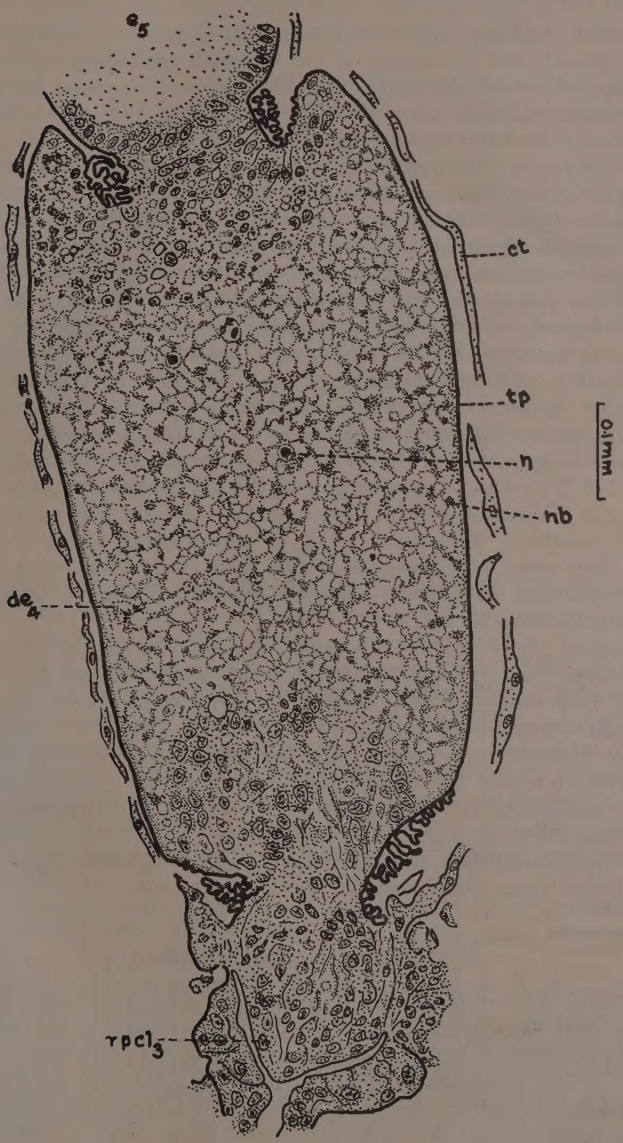
A similar process is repeated when the third egg is discharged and its corpus luteum is formed above the disintegrating remains of the two preceding ones (fig. 18). In such a conglomeration of successively formed corpora lutea the cavity of the first is seen in a state of extreme degeneration, filled with distorted cells and about to be obliterated. On account of the relatively rapid degeneration of the corpora lutea, more than three are never distinguished in the same section.

The *tunica propria* continues folding in the way described earlier. It seems quite likely that the folding might, in fact, be the mechanical cause of ovulation or at least contribute towards it, but this is not certain. At any rate the simple primary folds become converted into secondary formations and finally into an irregular folded mass which materially adds to the external diameter at the base of the ovariole.

FIGS. 18–19.—(18) L.S. through base of ovariole within 24 hours after laying third pod, showing three corpora lutea. (19) L.S. through base of partially degenerate ovariole showing resorption body formed by resorption of $egg_1$, while $egg_2$ is still immature and without chorion.




FIGS. 20–21.—(20) L.S. through base of partially degenerate ovariole, showing two successive resorption bodies. (21) L.S. through base of partially degenerate ovariole, showing much condensed resorption body formed by resorption of $egg_1$ and having mature $egg_2$ with chorion.




22

Fig. 22.—L.S. through base of ovariole after laying fourth pod, showing degeneration of the fairly long basal egg forming resorption body.

### (5) *Degenerate Ovarioles and the Formation of Resorption Bodies*

Sections of the basal pigmented region of the degenerate ovarioles referred to earlier reveal that the nuclei of the follicular epithelium are undergoing necrobiosis and are less basophilic (fig. 19) than in the corpus luteum of the normal ovarioles. The *tunica propria* is folded as in the normal corpus luteum, indicating that the basal region of the degenerate ovariole has originated from a follicle which was once larger. Since counts of eggs laid show that no egg was ovulated from these ovarioles it would appear that the basal oocyte, instead of developing normally, has undergone resorption to form a pigmented corpus-luteum-like structure. Thus I conclude that a structure similar to a true corpus luteum may also be formed when the oocyte is resorbed in the follicle of the young ovarioles before any ovulation has occurred, but the structures formed in this way are always pigmented. They are hereafter referred to as resorption bodies to distinguish them from the true corpora lutea developed after ovulation. Sometimes two consecutive oocytes may undergo resorption at the base of an ovariole, and their pigmented resorption bodies are formed one after the other, separated by a well defined constriction (fig. 20). Histological examination of the folding of *tunica propria*, the degree of degeneration of the follicular epithelium and the different intensities of pigment colours in the proximal and distal regions serve to demonstrate that two oocytes have been resorbed. Such degeneration of the ovarioles is not a permanent feature. They may later become normal and after ovulation a colourless corpus luteum may be formed above the remains of the previously formed resorption body (figs. 11, 21).

Histological study of the subnormal ovarioles distinguished above was unsatisfactory owing to the difficulty of cutting sections of some of the yolky material. Such observations as were made, however, on insects reared under adverse experimental conditions or those after the fourth or fifth ovulation, showed considerable degeneration. The follicular epithelium was disorganised, the yolk resorbed and the cavity filled with apparent vacuoles and coarse basophilic aggregations of chromatin (fig. 22). The *tunica propria* of such a degenerating follicle is folded at the proximal and distal ends. As in the resorption bodies, therefore, the deposition of pigment is related to the degeneration of a follicle from which ovulation has not taken place.

## IV. *Schistocerca gregaria* (Forskal)

Many workers have reported yellow ovariole plugs in *Schistocerca gregaria* but no detailed study seems to have been made. The following observations suggest that conditions are very similar to those found in *Locusta*.

In one set of experiments, five mating pairs of known previous history were kept in celluloid breeding cages as in *Locusta*, at 29° C. and 55 per cent. R.H. during the night and 36° C. and 40 per cent. R.H. in the day. After the first pod was laid the insect was dissected. The eggs laid in the pod were counted and the ovarioles with colourless corpora lutea and those with pigmented plugs divided into categories as before. It was observed that: (1) the number of eggs in a pod corresponds exactly with the number of ovarioles possessing colourless corpora lutea, just as in *Locusta*, and (2) there are some partially

degenerate ovarioles with pigmented plugs. The histology of the pigmented plug and the normal corpus luteum is similar to the resorption body and the colourless corpus luteum in *Locusta*.

In another set of experiments, five recently emerged females were kept in five cages as above, and no male was given to pair. The dissection was done as soon as the first pod was laid. The eggs were counted and the ovarioles divided into categories, as in the previous experiment. It was found that (1) the number of eggs in the pod corresponds exactly with the number of ovarioles possessing colourless corpora lutea, and (2) the number of degenerate ovarioles with pigmented resorption bodies is greater than in fertilised females.

Histological study of these insects showed the pigmented structure to be a well developed resorption body resembling that of *Locusta* but it was accompanied by degeneration of the adjacent egg, which was also pigmented.

From the above information, it may reasonably be concluded that in *Schistocerca*, also, the appearance of pigment at the base of the partially degenerate ovariole is associated with resorption of the basal egg, with or without accompanying degeneration of the next youngest egg. These resorptive processes are more extensive in unmated females, apparently because in them oviposition is delayed.

## V. Summary

1. Mated females of *Locusta migratoria* usually lay their first egg-pod between nine and sixteen days after emergence and subsequent pods at intervals of two to six days. Each pod contains 50–100 eggs.

2. Immediately before oviposition, a single egg is ovulated from most of the ovarioles. A few ovarioles produce no egg but may become functional at a later ovulatory cycle. Conversely, a few ovarioles ovulating at one cycle do not produce an egg at the next one.

3. Under normal circumstances, ovulation is accompanied or immediately followed by folding of the *tunica propria* around the ovulating follicle, followed within 24 hours by disorganisation of the follicular epithelium, karyolysis, and progressive contraction of the *tunica propria* through the formation of secondary folds.

4. The resulting corpus luteum is not pigmented and up to three corpora lutea, formed after successive ovulations, may be recognised histologically in older females. Their detailed structure is described.

5. Pigmented (orange-yellow) structures at the base of the ovariole are infrequent under satisfactory environmental conditions. They are of two quite different types ; (*a*) resorption bodies formed when the basal oocyte is resorbed instead of being ovulated ; and (*b*) corpora lutea formed in the normal way but later acquiring pigment through partial resorption of the adjacent oocyte. The formation of the pigmented corpus luteum (type *b*) could be induced experimentally by keeping females under adverse conditions, which tended to promote some degree of resorption. Some variation of the two main types of pigmented structures are discussed.

6. A small number of observations on *Schistocerca* suggest that it resembles *Locusta* in ovulatory behaviour and the formation of corporea lutea and resorption bodies.

## VI. Acknowledgments

I am deeply indebted to Prof. O. W. Richards for his invaluable guidance and encouragement throughout this investigation and also for going through the manuscript. I am also grateful to Mr. R. G. Davies and Dr. K. Boratynski for their assistance.

## VII. References

Ahrens, W., 1935, Die Entwicklung des "Corpus luteum" bei den Insekten. Nach Untersuchungen an *Termes redemanni*. *Z. mikr-anat. Forsch.* **37**: 467–500.

Bonhag, P. F. and Wick, J. R., 1953, The functional anatomy of the male and female reproductive systems of the Milkweed Bug, *Oncopeltus fasciatus* (Dallas)—(Heteroptera Lygaeidae). *J. Morph.* **93**: 177–283.

De Lerma, B., 1951, Ricerche istochimiche sui "Corpi lutei" di Ortotteri con l'applicazione del test di schultz per le sostanze colesteroliche. *Boll. Zool. Torino* **18**: 5–6.

Iwanoff, P. P. and Mestcherskaja, K. A., 1935, Die physiologischen Besonderheiten der geschlechtlich unreifen Insecktenovarien und die Zyklischen Veränderungen ihrer Eigenschaften. *Zool. Jb.* (*Zool.*) **55**: 281–348.

Palm, N. B., 1948, Normal and pathological histology of the ovaries in *Bombus* Latr. (Hymenopt.). *Opusc. Ent. Lund., Suppl.* **7**: 1–101.

Pantin, C. F. A., 1948, *Notes on microscopical technique for zoologists*. Cambridge.

Phipps, J., 1949, The structure and maturation of the ovaries in British Acrididae. *Trans. R. ent. Soc. Lond.* **100**: 233–47.

—— 1950, The maturation of the ovaries and the relation between weight and maturity in *Locusta migratoria migratorioides* (R. and F.). *Bull. ent. Res.* **40**: 539–57.

Roonwal, M. L., 1947, On variation in the number of ovarioles and its probable origin in the desert locust, *Schistocerca gregaria* (Orthoptera, Acrididae). *Rec. Indian Mus.* **44**: 375–84.

Vel'tischev, P. A., 1941, New data on bio-ecology of the Asiatic locust in the Amu-Daryo delta. *Priroda* **1941**: 78–81.

Wigglesworth, V. B., 1936, The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Quart. J. micr. Sci.* **79**: 91–121.

Wright, G. P., 1954, *An introduction to Pathology*. 2nd Edn. London: 166–72.

## Key to Lettering of Figures

*c*, chorion.
*cl*, corpus luteum.
*cm*, compact chromatin transforming into mauve globule.
*ct*, connective tissue sheath.
*c.c.f*, coarse chromatin fragments.
*c.cl*, colourless corpus luteum.
$de_4$, degenerating fourth egg.
$e_2$, $egg_2$.
$e_3$, $egg_3$.
$e_4$, $egg_4$.
$e_5$, $egg_5$.
$fO_2$, follicle of second egg.
$l_1$, lumen of first follicle.
$l_2$, lumen of second follicle.
$l_3$, lumen of third follicle.
*lo*, lobe of pyknotic follicular tube.
*m.g.v*, mauve globule in vacuole.
*mg*, mauve globule.
*n*, necrotic follicle nuclei.
*nb*, necrobiotic follicle nuclei.
*p.cl*, pigmented corpus luteum.
*pe*, pedicle.
*pk*, pyknotic cell.
*pg*, passage of egg.
*r.pi*, ring of pigment.
*rb*, resorption body.
$r.p.cl_3$, remains of preceding three corpora lutea.
$rb_1$, first resorption body.
$rb_2$, second resorption body.
*tp*, tunica propria.
*v*, vacuole.
'*x*', free chromatin granules